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Nuclear genes, *matK* and the phylogeny of the Poales

Anne Hochbach,[1] H. Peter Linder[2] & Martin Röser[1]

1 *Institute of Biology, Department of Systematic Botany, Martin-Luther-University Halle-Wittenberg, Neuwerk 21, 06108 Halle (Saale), Germany*

2 *Department of Systematic and Evolutionary Botany, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland*

Authors for correspondence: *Anne Hochbach, anne.hochbach@botanik.uni-halle.de; Martin Röser, martin.roeser@botanik.uni-halle.de*

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Abstract Phylogenetic relationships within the monocot order Poales have been well studied, but several unrelated questions remain. These include the relationships among the basal families in the order, family delimitations within the restiid clade, and the search for nuclear single-copy gene loci to test the relationships based on chloroplast loci. To this end two nuclear loci (*PhyB*, *Topo6*) were explored both at the ordinal level, and within the Bromeliaceae and the restiid clade. First, a plastid reference tree was inferred based on *matK*, using 140 taxa covering all APG IV families of Poales, and analyzed using parsimony, maximum likelihood and Bayesian methods. The trees inferred from *matK* closely approach the published phylogeny based on whole-plastome sequencing. Of the two nuclear loci, *Topo6* supported a congruent, but much less resolved phylogeny. By contrast, *PhyB* indicated different phylogenetic relationships, with, inter alia, Mayacaceae and Typhaceae sister to Poaceae, and Flagellariaceae in a basally branching position within the Poales. Within the restiid clade the differences between the three markers appear less serious. The *Anarthria* clade is first diverging in all analyses, followed by Restionoideae, Sporadanthoideae, Centrolepidoideae and Leptocarpoideae in the *matK* and *Topo6* data, but in the *PhyB* data Centrolepidoideae diverges next, followed by a paraphyletic Restionoideae with a clade consisting of the monophyletic Sporadanthoideae and Leptocarpoideae nested within them. The Bromeliaceae phylogeny obtained from *Topo6* is insufficiently sampled to make reliable statements, but indicates a good starting point for further investigations. We find that *matK* is remarkably good at retrieving the chloroplast phylogeny, that *Topo6*, despite low resolution, is suitable to test the generality of the plastid phylogeny as a taxic phylogeny, that *PhyB* might be too complex to be really useful at the level of families within an order, that the inclusion of the centrolepids in Restionaceae might be valid, but that there is no phylogenetic support for or against including the *Anarthria* clade in Restionaceae. The basal arrangement of families in the Poales (Bromeliaceae, Typhaceae, Rapateaceae) remains unresolved.

Keywords Bromeliaceae; *matK*; molecular phylogeny; nuclear loci; Poales; Restionaceae

Supplementary Material Electronic Supplement (Figs. S1, S2) and DNA sequence alignments are available from https://doi.org/10.12705/673.5.S1 and https://doi.org/10.12705/673.5.S2, respectively.

■ INTRODUCTION

The large order Poales is recognized by the Angiosperm Phylogeny Group IV (APG IV, 2016) within the monocots. Poales comprise approximately 20,000 species, thus 7% of all angiosperms and about one-third of monocots (Givnish & al., 2010; Bouchenak-Khelladi & al., 2014). Fourteen families are currently recognized, ranging in size from the Poaceae with ca. 12,000 species, to the medium-sized Cyperaceae (ca. 5500 species) and Bromeliaceae (3475 species), to the small families Flagellariaceae, Joinvilleaceae and Thurniaceae (4 species each) (Christenhusz & Byng, 2016). Poales are morphologically and biologically enormously variable and present in almost all terrestrial and many aquatic ecosystems. Poaceae is arguably the most successful angiosperm family (Linder & al., 2018): grass species dominate the savannas and steppes, thus ca. 30% of the global vegetated land surface, they fuel frequent fires which lead to extensive deforestation (Bond & Midgley, 2012), and grasses have provided the food base for a large radiation of grazers. Grassland provided the ecological context for human evolution, and currently wheat, rice and maize provide about half of the global plant-derived food energy (cf. Givnish & al., 2010; Bouchenak-Khelladi & al., 2014; Kellogg, 2015).

The grouping of families that make up the current Poales was first proposed by Duvall & al. (1993), based on *rbcL* sequence data. This was combined with a morphological dataset by Kellogg & Linder (1995). These families were grouped into the currently circumscribed Poales by APG (1998) and further elaborated by Chase & al. (2000). Bremer (2002) explored the detailed phylogenetic relationships among these families, based on chloroplast DNA data. The relationships among the Poales families can be simplified to five groups/clades (Linder & Rudall, 2005). The paraphyletic basal Poales includes Bromeliaceae, Rapateaceae and Typhaceae. The four other clades are termed "core Poales". Cyperaceae, Juncaceae and Thurniaceae form a phylogenetically robust cyperid clade. This is sister to the xyrid clade, which comprises Eriocaulaceae, Mayacaceae and Xyridaceae, and which is the

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most ambiguous clade in terms of circumscription and phylogenetic position. The restiid clade contains Anarthriaceae, Centrolepidaceae and Restionaceae, and this is sister to the graminid clade. The graminid clade (Ecdeiocoleaceae, Flagellariaceae, Joinvilleaceae, Poaceae) reflects a relatively recent circumscription, with Ecdeiocoleaceae as sister to Poaceae. The phylogeny based on 81 plastid genes of Givnish & al. (2010) confirmed these systematic relationships, but with the xyrids paraphyletic, and the basal group arranged as (Bromeliaceae, (Typhaceae, (Rapateaceae, (the rest)))). The largest phylogenetic analysis of Poales to date was conducted by Bouchenak-Khelladi & al. (2014), based on *rbcL* and *ndhF* sequences for 545 species. The cyperid clade there contains additionally Rapateaceae and Mayacaceae, but this rearrangement is not strongly supported. The remaining families in the xyrid clade are Eriocaulaceae and Xyridaceae. Phylogenetic studies using genome structural, plastid and/or mitochondrial data (cf. Duvall & al., 1993; Bremer, 2002; Michelangeli & al., 2003; Davis & al., 2004; Graham & al., 2006; Givnish & al., 2010; Bouchenak-Khelladi & al., 2014) thus converge on a (largely) common phylogenetic hypothesis for the Poales.

This "common phylogenetic hypothesis" is based largely on the chloroplast genome. Whether this is congruent with the phylogeny (or phylogenies) of the nuclear genome can only be tested by phylogenies derived from the separate genomes. Although Chase & al. (2006) also used 26S rDNA, this was only a minor component of the dataset. The first monocot study based on a nuclear low-copy gene (*PhyC*), as well as a combined dataset (Hertweck & al., 2015), indicated an increase in the backbone support values along the tree, but did not report on the relationships among the families within the Poales. Barrett & al. (2016) analyzed a matrix of 75 protein coding genes of the plastome, and although they retrieved the graminid, restiid and cyperid clades, the members of the xyrid clade were in a paraphyletic arrangement. The basal group was construed as (Bromeliaceae, (Typhaceae, (Rapateaceae, (Mayacaceae, (the rest))))). A concatenated and coalescence-based analysis of 234 single-copy orthogroups (McKain & al., 2016), nuclear-encoded, and so occasionally with orthology/paralogy issues, found a similar result, but with the basal group assembled as (Typhaceae, (Bromeliaceae, (Rapateaceae, (cyperids, (paraphyletic xyrids, (restiids, graminids)))))). This shows that although the cyperids, restiids and graminids as well as the basal group are robustly retrieved, there is still no robust result for the xyrids (specifically for the placement of Mayacaceae), or for the sequence of families within the basal group.

The phylogenetic relationships within the larger families in the Poales have received substantial attention, but almost only based on plastid sequence variation. In Poaceae, in addition to several chloroplast markers (e.g., *ndhF*, *rpoC2*, *matK*) and recent plastome analyses (cf. Burke & al., 2016; Teisher & al., 2017), also *waxy* (Mason-Gamer & al., 1998), *PhyB* (Mathews & al., 2000) and ITS (Hsiao & al., 1999) have been used. In addition, although the phylogeny of the Bromeliaceae as a whole is based on chloroplast data (Givnish & al., 2011), Barfuss & al. (2016) included the nuclear encoded *PhyC* in their analysis of the subfamily Tillandsioideae. In Restionaceae (Briggs & al., 2000; Hardy & al., 2008; Bouchenak-Khelladi & Linder, 2017) and Cyperaceae (Muasya & al., 2009) generic and species-level phylogenies have been based solely on chloroplast DNA sequence data. Although Litsios & al. (2014) also used *PhyB* in the African Restionaceae, they did not analyze the datasets separately, thus not providing insights into how congruent the plastid and nuclear datasets are. In the few instances where nuclear data were used, they were usually ITS (for example, in Juncaceae; Záveská-Drábková, 2008), which has its own special problems, such as orthology/paralogy conflation, compensatory base changes, problems in alignment due to indel accumulation, sequencing errors, or some combination of these phenomena (cf. Álvarez & Wendel, 2003 and references therein; Nieto Feliner & Rosselló, 2007). Consequently, it remains unclear how congruent chloroplast and nuclear sequence data may be in these families.

Here, we first test whether *matK* retrieves the same phylogeny as the full chloroplast genome. As the chloroplast is inherited as a complete unit, we expect that this should be the case, but that the topology should receive less support or be less resolved, due to the smaller number of informative sites. The chloroplast *matK* region already led to meaningful results in previous phylogenetic analyses of monocots (e.g., Tamura & al., 2004; Givnish & al., 2010; Hertweck & al., 2015) and parts of the order Poales, such as the graminid clade (Marchant & Briggs, 2007), Bromeliaceae (Evans & al., 2015), the cyperid genus *Carex* L. (Gebauer & al., 2014), Restionaceae (Hardy & al., 2008) and Poaceae (Hilu & al., 1999; Blaner & al., 2014; Hochbach & al., 2015).

Second, we ask whether the nuclear data support the plastid phylogeny (either whole or partial plastomes or *matK* only), both at the family level, and within the Restionaceae and Bromeliaceae. The discrepancies between the transcriptome dataset of McKain & al. (2016) and the plastid trees suggest that there might be differences. Consequently, we sequenced two nuclear loci (*PhyB*, *Topo6*, see Hochbach & al., 2015) and analyzed them separately. To evaluate the utility of these markers for phylogenetic studies at different systematic levels, the restiid clade and Bromeliaceae were additionally investigated. Over the past several years phylogenetic investigations based on nuclear regions have become more and more important for plant systematics (Zimmer & Wen, 2012). To date, the nuclear single-copy gene topoisomerase 6 (*Topo6*; cf. Hartung & al., 2002; Blattner, 2016) has only been used to address questions about organismal relationships within Poaceae (Blaner & al., 2014), the subfamily Pooideae (Hochbach & al., 2015), the Aveneae/Poeae tribe complex (Wölk & Röser, 2014, 2017; Wölk & al., 2015) and the genus *Hordeum* L. (Brassac & al., 2012; Brassac & Blattner, 2015). Characteristics, problems and phylogenetic usefulness of single-copy genes and especially of *Topo6* have been discussed in detail by Blattner (2016). The second nuclear marker region investigated in this study is a part of the phytochrome B gene (*PhyB*). It is present in all seed plants and has already proved to be useful in angiosperms (Mathews & al., 1995), palms (Ludeña & al., 2011), Restionaceae (Litsios & al., 2014) and Poaceae (Mathews & al., 2000).

■ MATERIALS AND METHODS

Taxon sampling. — In order to test whether *matK* retrieves the same phylogeny as that inferred from whole-plastome sequence data, we sampled all families in the Poales, each represented by several phylogenetically dispersed species. In total, we included 148 species of Poales representing the 14 recognized families (APG IV, 2016). We also included, as outgroups, *Wendlandiella gracilis* Dammer of Arecales and *Musa velutina* H.Wendl. & Drude of Zingiberales, as these commelinid orders are closely related to Poales (Givnish & al., 2010). Plant material for the molecular phylogenetic analyses was taken from silica gel-dried leaf material collected in the field, from the living plant collection of the Halle Botanical Garden or from leaves of herbarium specimens. Taxa, authorities and GenBank accession numbers are listed in Appendix 1, together with brief voucher data for new sequences.

Molecular methods. — Qbiogene FastPrep FP120 cell disrupter (Heidelberg, Germany) was used to homogenize 20–45 mg leaf tissue per sample. Extraction of total genomic DNA was conducted with the NucleoSpin Plant Kit in accordance to manufacturer's protocol (Macherey-Nagel, Düren, Germany). The concentration of the DNA samples was checked with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). The plastid *matK* gene and the two nuclear single-copy gene regions *Topo6* exon 8–11 and *PhyB* were PCR-amplified for the phylogenetic analyses. PCR modifications and sequencing followed methods described by Blaner & al. (2014) and Hochbach & al. (2015). The forward and reverse primers used in this study are listed in Table 1. Cloning of PCR products, which had ambiguous peaks or too low DNA concentration for direct sequencing, was carried out using the pGEM-T Easy Vector System of Promega (Mannheim, Germany). Following the technical manual, the relevant purified amplicons were ligated and transformed. For each cloned species and marker region up to 40 colonies were selected. The presence of the insert for the single picked colonies was checked by PCR. From these clone colonies, 20 positively tested untreated PCR products were sent to LGC Genomics (Berlin, Germany) for purification and sequencing.

Data and phylogenetic analyses. — The chromatograms of forward and reverse sequence segments were manually edited and aligned using Sequencher 4.10.1 (Gene Codes, 2010). Ambiguous alignment positions were coded according to the

Table 1. Primers used for amplification and sequencing of the chloroplast and nuclear regions.

DNA region	Primer name	Primer sequence 5′→3′	Reference
matK	5S-1F	ACC CTG TTC TTGA CCA TAT TG	Hilu & al. (1999)
	PO-matK 620R	CGC AAT AAA TGC AAA GA(CT) GGA AC	Döring & al. (2007)
	PO-matK 470F	CCA AAG TTT CAG AAT TTA CGC TCT ATT C	Schneider & al. (2009)
	PO-matK 1070R	CCA GCA TTT GAT TCC TTA	Schneider & al. (2009)
	PO-matK 860F	CAT TAT GTT CGA TAT CAA GG	Schneider & al. (2009)
	PO-matK 1420R	TTA CGA GCT AAA GTT CTA GC(AG) CA	Döring & al. (2007)
	PO-matK 1300F	TCA GAT TGG GAT ATT CTT GAT CG	Döring & al. (2007)
	trnK-2R	AAC TAG TCG GAT GGA GTA G	Johnson & Soltis (1994)
	PO-matK 1900F	ACA TAG GGA AAG TCG TGT GC	Schneider & al. (2012)
	psbA-R	CGC GTC TCT CTA AAA TTG GAG TCA T	Johnson & Soltis (1994)
Topo6 exon 8–11	Topo6P_8_700F	GCA CTT ATW TGG TCA AAG ATG AG	Blaner & al. (2014)
	Topo6P_11_1030R	AGG AGG CAT AAC ATC TGT C	Blaner & al. (2014)
	Topo6_8_Poales_F	GGT CWA AGA TGA GCA CHG GNC TTC C	This study
	Topo6_11_Poales_R	GGA GGC ATW ACA TCT GTY CKT CG	This study
	Topo6_9_Poales_F	TGG CAT GGN GCW GAG ATT CAW GT	This study
	Topo6_9_Poales_R	ACW TGA ATC TCW GCN CCA TGC CA	This study
PhyB	B-up	GAG CCN GCY MGH ACS GAR GAY CC	Mathews & al. (2000)
	B-down	GCR TCC ATY TCK GCA TTY TCC CA	Mathews & al. (2000)
	gymfor	CAR TAY ATG GCN AAY ATG G	Mathews & al. (2000)
	B_down_Poales	GCA TTY TCC CAV GGC AAG CTC CT	This study
	B_up_Poales	TCC CAG AAG CTY GCC GTS CGS GCC AT	This study
	PHYB_mid_F	CTS CAR CTB AAC ATG GAR CT	This study
	PHYB_mid_R	AGY TCC ATG TTV AGY TGS AG	This study
	PHYB_cyp_mid_F	GGB ATH GTB ACN CAR AGY CC	This study
	PHYB_cyprids_R	GGG TGC ATN CKY TGS CCR TC	This study
	PHYB_Poales_2F	CGC TCC CYG GYG GNG ACR TC	This study
	PHYB_Poales_2R	CYT CYG GGT GRT GCT THG C	This study

IUPAC Code, and whole equivocal regions were excluded from the analyses. To reduce the number of singletons in the alignment, highly similar sequences of individual clones were summarized to consensus sequences (cf. Brassac & al., 2012). Two *matK* datasets were assembled: a complete dataset, including all samples sequenced, and a reduced dataset, including only these species also sequenced for the nuclear loci. Potentially informative indels were scored only for the complete Poales *matK* alignment following the "simple indel coding" method (Simmons & Ochoterena, 2000) and added as binary presence/absence characters to the matrix.

In order to test for analytical artifacts, the datasets were analyzed using three methods of phylogenetic inference: Bayesian inference (BI) implemented in MrBayes v.3.2.6 (Ronquist & al., 2012), maximum likelihood (ML) implemented in raxmlGUI (Stamatakis, 2006; Silvestro & Michalak, 2012), and maximum parsimony (MP) implemented in PAUP* v.4.0b10 (Swofford, 2002). For the complete and the reduced *matK* Poales datasets, the GTR+I+Γ substitution model was selected as best-fit by the Akaike information criterion (AIC) implemented in MrModeltest v.2.3 (Nylander & al., 2004). The optimal model identified for the congruent *Topo6* Poales and for the congruent *PhyB* Poales datasets was HKY+I+Γ, and for the *matK* and *PhyB* datasets GTR+Γ, and for the *Topo6* Restionaceae and *Topo6* Bromeliaceae datasets HKY+Γ.

BI analyses were performed with two sets of four chains, each run for 20 million generations, and trees sampled and saved every 1000th generation. All split frequencies were less than 0.01 and a majority-rule consensus tree was calculated for each dataset after discarding the first 25% of the trees as "relburn-in". Posterior probabilities (PP) of 0.95–1.00 were considered significant. ML analyses were based on rapid bootstrap analysis, searching for best-scoring ML trees over 1000 bootstrap (BS) replicates. Heuristic tree searches for MP analyses were conducted with all characters and character-state changes equally weighted, TBR branch swapping, maxtrees limited to 10,000 and replicated for 200 random taxon additions. The set of most parsimonious trees was summarized into a strict consensus tree. For bootstrap analyses following options were set up to test the statistical support of clades: 500 replicates, 1000 maxtrees, TBR, and closest addition. The consistency index (CI; Kluge & Farris, 1969), retention index (RI; Farris, 1989) and other tree statistics were also calculated using PAUP* v.4.0b10. Problems such as conflicting relationships in the trees obtained from individual analyses were treated following Hochbach & al. (2015).

In the following, the terms weak, moderate and strong (cf. high) were used for bootstrap values of 50%–74%, 75%–90% and 91%–100%, respectively.

Checking *PhyB* data. — In order to confirm that anomalous phylogenetic results for some of the Poales species based on the *PhyB* dataset are not due to the presence of several non-homologous copies, we cloned the relevant species (*Mayaca fluviatilis* Aubl. [Mayacaceae], *Eriocaulon scariosum* Sm., *Leiothrix flavescens* (Bong.) Ruhland [both Eriocaulaceae], *Flagellaria indica* L. [Flagellariaceae] and *Typha* ×*glauca* Godr. [Typhaceae]). Furthermore, an additional accession of *Mayaca fluviatilis* was also sequenced.

■ RESULTS

Order Poales (*matK*, *Topo6*, *PhyB*). — The complete dataset of the *matK* gene contained 142 sequences. After inclusion of 19 indels the final matrix comprised 1762 characters. Of 1376 variable characters, 1071 (61%) were parsimony-informative. The heuristic search of the MP analysis yielded >10,000 trees of 6430 steps, a CI of 0.396 and RI of 0.809 (Table 2). The three phylogenetic algorithms (BI, ML, MP) resulted in similar tree topologies, therefore only the Bayesian 50% majority-rule consensus tree is shown (Fig. 1). The graminids, restiids and cyperids are each retrieved as monophyletic. Xyrids are paraphyletic, with Mayacaceae sister to xyrids s.str., graminids and restiids. In the basal group (Bromeliaceae, Typhaceae, Rapateaceae), Bromeliaceae and Typhaceae are in a basal polytomy. In total, 71% of the 14 nodes had more than 70% bootstrap support. All families are highly supported as monophyletic.

In the reduced *matK* datasets, and *Topo6* and *PhyB* datasets, each dataset comprised 52 ingroup taxa. Unfortunately, it was impossible to amplify *Topo6* and *PhyB* sequences for

Table 2. Comparison and summary of maximum parsimony-obtained tree statistics of all datasets used.

Characteristic	*matK* Poales	*matK* Poales congruent	*Topo6* Poales congruent	*PhyB* Poales congruent	*matK* Restionaceae	*Topo6* Restionaceae	*PhyB* Restionaceae	*Topo6* Bromeliaceae
Ingroup taxa	140	52	52	52	19	19	19	20
Consistency index	0.396	0.497	0.408	0.326	0.732	0.772	0.665	0.830
Retention index	0.809	0.738	0.629	0.607	0.724	0.703	0.569	0.689
Base pairs	1762	1665	347	1377	1559	2452	1162	1022
Parsimony informative	1071 (61%)	802 (48%)	161 (46%)	511 (37%)	381 (24%)	263 (11%)	329 (28%)	147 (14%)
Trees	>10,000	24	>10,000	2	6	102	2	180
Tree length	6430	3619	855	3657	1208	911	1348	690
Excluded characters	–	–	all introns	923	–	125	–	–
Substitution model	GTR+I+Γ	GTR+I+Γ	HKY+I+Γ	HKY+I+Γ	GTR+Γ	HKY+Γ	GTR+Γ	HKY+Γ

Thurniaceae, so only 13 of 14 Poales families are represented in these datasets. All analyses (BI, ML, MP) of each dataset led to similar tree topologies and therefore only the Bayesian 50% majority-rule consensus trees are shown in Fig. 2 (detailed trees for *Topo6* and *PhyB* in the Electr. Suppl., Figs. S1 & S2).

Several problems were noticed during laboratory work on the single-copy nuclear loci. There is a very large insertion in intron 8 of *Topo6* in the African Restionaceae, which is not present in Australian species. Likewise, there are immense insertions in the two introns 8 and 9 of *Topo6* within Cyperaceae and Juncaceae. These insertions are very difficult to amplify and sequence. The amplification of *PhyB* in Bromeliaceae was also complicated. The introns of *Topo6* are only alignable at and below the family level.

The *matK* alignment consisted of 1665 base pairs, 802 (72%) of 1109 variable characters were parsimony-informative and the heuristic search of the MP analysis provided 24 most parsimonious trees with a length of 3619 steps (CI = 0.497, RI = 0.738; cf. Table 2). This *matK* tree corresponds to the complete *matK* dataset of Poales shown in Fig. 2. The only

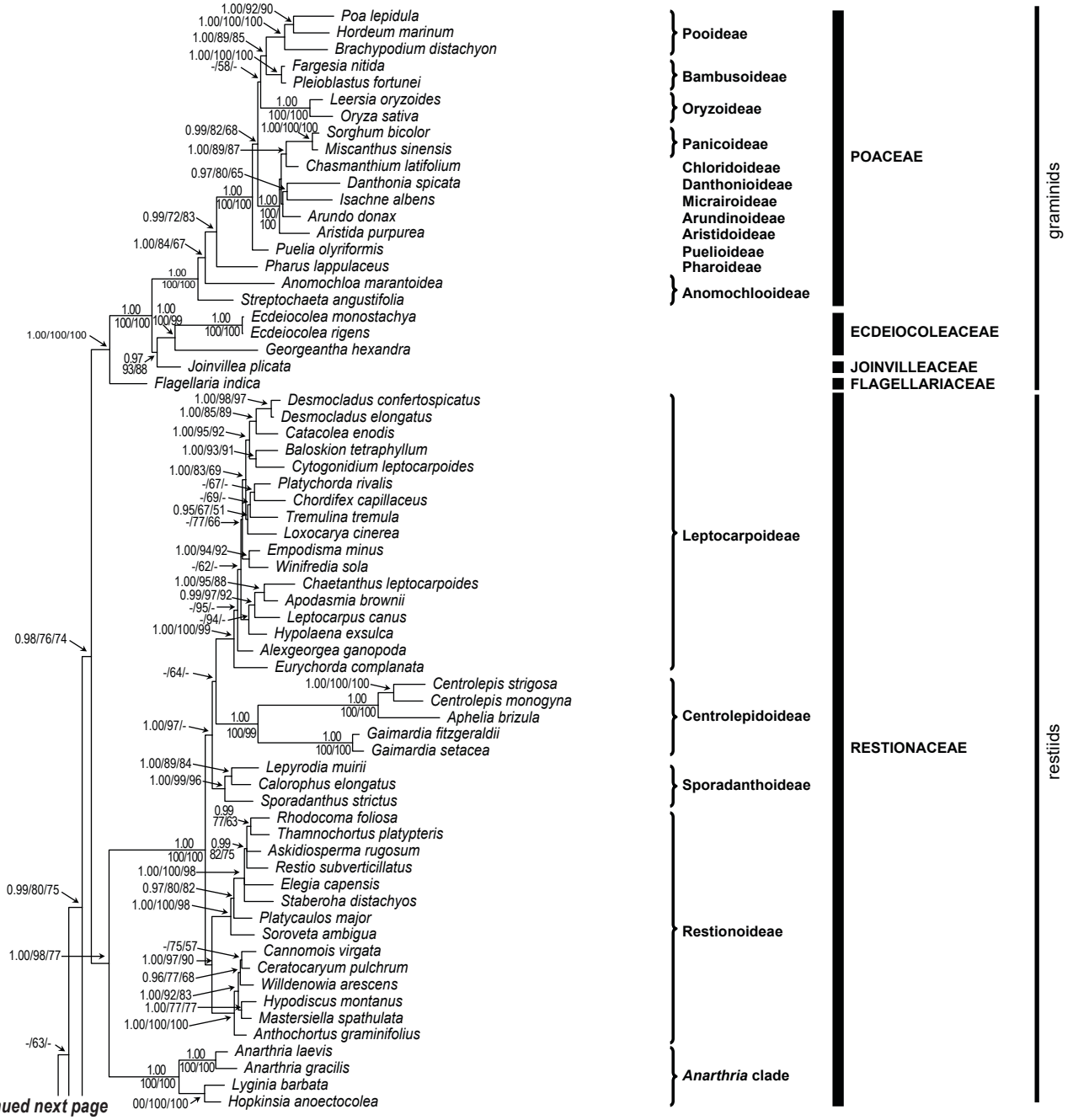
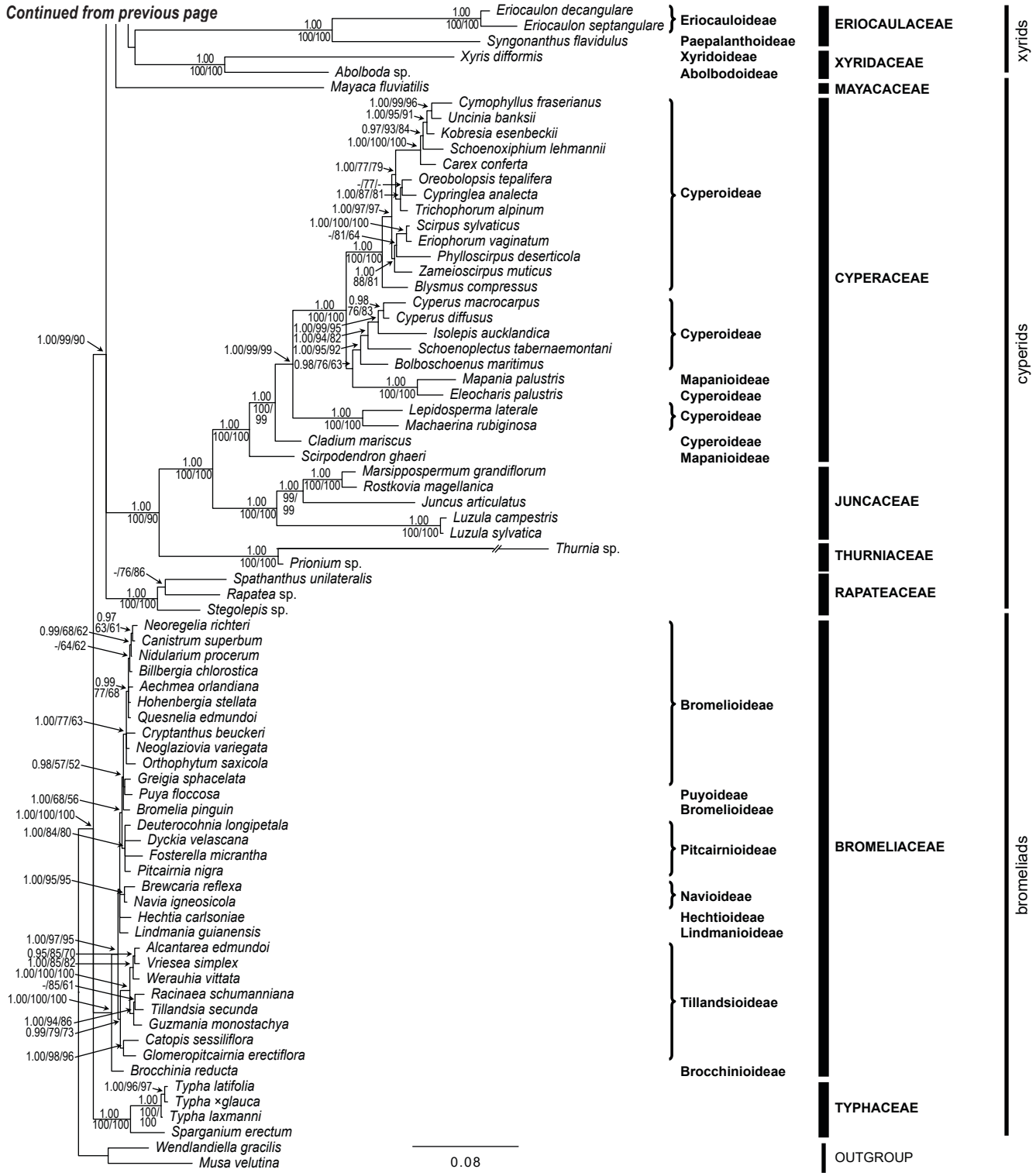


Fig. 1. Bayesian 50% majority-rule consensus tree obtained from chloroplast *matK* gene DNA sequences in representatives of Poales. Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS).

difference is the weakly supported node (ML-BS 73%, MP-BS 65%) combining cyperids, xyrids and restiids, where for the complete dataset a polytomy of Rapateaceae, cyperids and (xyrids, restiids, graminids) is retrieved (Fig. 1).

After the exclusion of all introns, which could not be aligned, the *Topo6* matrix comprised 347 characters. Of 207 variable characters, 161 (78%) were parsimony informative and more than 10,000 trees of 855 steps (CI = 0.408; RI = 0.629; Table 2) were found. The backbone of the *Topo6* tree is poorly resolved (Electr. Suppl.: Fig. S1). Bromeliaceae (ML-BS 57%, MP-BS 71%) and Poaceae (PP 0.98, ML-BS 65%, MP-BS 51%, hereafter when all three support values were calculated they



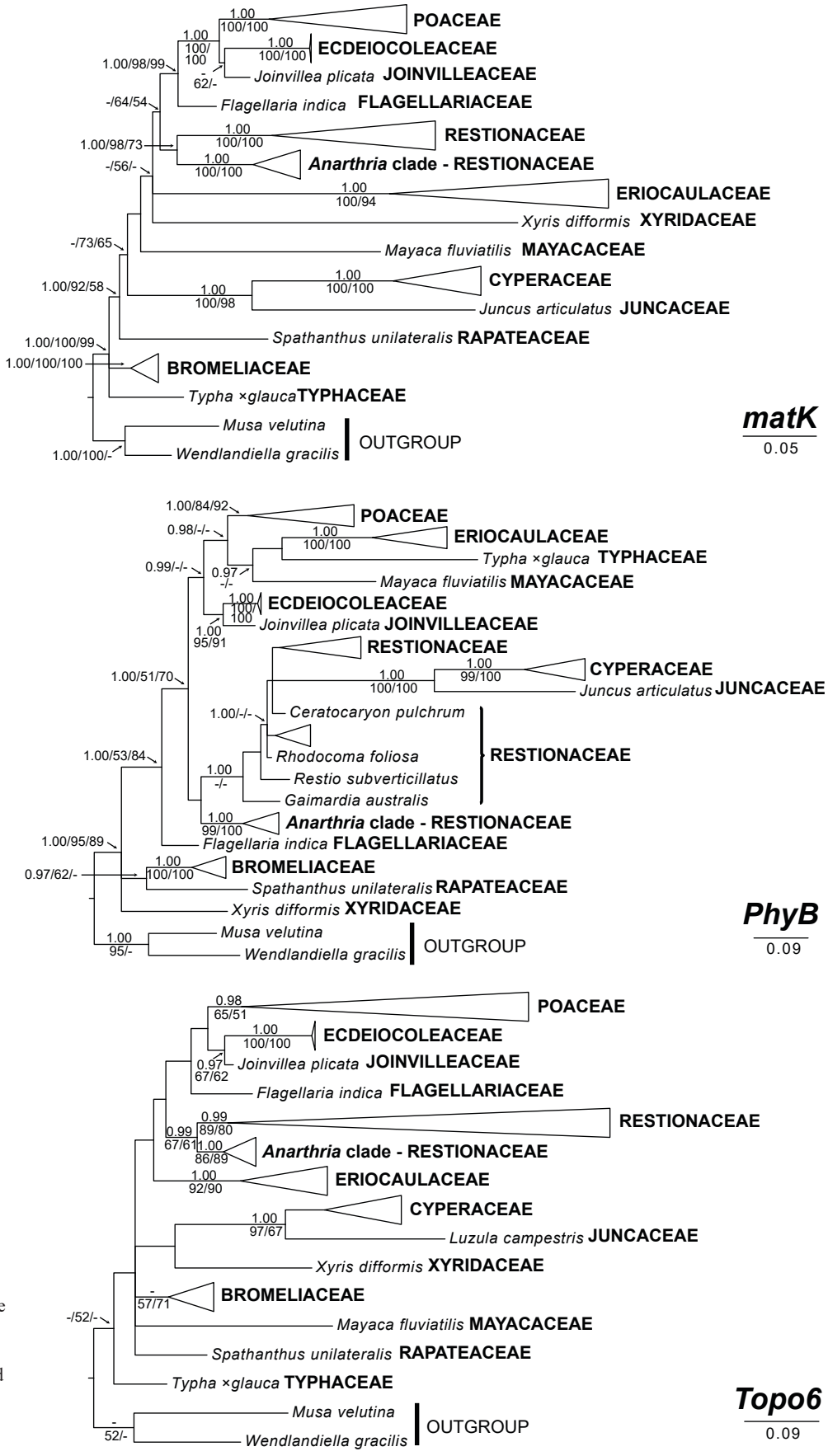


Fig. 2. Simplified Bayesian 50% majority-rule consensus trees from individual loci sequences of congruent Poales sampling. Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS).

are reported in this sequence) are very weakly supported, but Eriocaulaceae (1.0, 92%, 90%), the *Anarthria* R.Br. clade representing the former family Anarthriaceae (1.0, 86%, 89%) and Ecdeiocoleaceae (1.0, 100%, 100%) strongly supported. The cyperids (1.0, 97%, 67%) and restiids (0.99, 89%, 80%) are retrieved, and the sister relationship between Joinvilleaceae and Ecdeiocoleaceae (0.97, 67%, 62%) is weakly to strongly supported.

The *PhyB* alignment encompassed a total of 1377 sites after the exclusion of 923 positions (733–827, 1105–1932) that could not confidently be aligned. Of 665 variable sites, 511 (77%) were parsimony informative. The heuristic search of the MP analysis yielded two most parsimonious trees with a length of 3657 steps (CI = 0.326, RI = 0.607; cf. Table 2). According to the *PhyB* phylogeny (Electr. Suppl.: Fig. S2) Xyridaceae and a weakly supported group encompassing Bromeliaceae and Rapateaceae (PP 0.97, ML-BS 62%) branch off first, followed by a moderately supported lineage (1.0, 53%, 84%) where *Flagellaria* is sister to the remaining Poales (1.0, 51%, 70%). The remainder formed a polytomy with strongly supported *Anarthria* clade (1.0, 99%, 100%) as sister to Restionaceae on the first branch. The second lineage (PP 1.0) includes Centrolepidoideae and a paraphyletic remaining Restionaceae with an embedded Cyperaceae (1.0, 99%, 100%) and Juncaceae united in a sister-group relationship (1.0, 100%, 100%). In the third group (PP 0.99) *Joinvillea* Gaudich. ex Brongn. & Gris is sister (1.0, 95%, 91%) to Ecdeiocoleaceae (1.0, 100%, 100%). Poaceae (1.0, 84%, 92%) is sister (PP 0.98) to a weakly supported clade (PP 0.97) with *Mayaca* Aubl., *Typha* L. and Ecdeiocoleaceae (1.0, 100%, 100%).

Restiid clade (*matK*, *Topo6*, *PhyB*). — The datasets of the restiid clade contain 19 ingroup and 3 outgroup taxa of the graminid clade. All analyses of each dataset resulted in highly similar tree topologies, so only the 50% majority-rule consensus trees of BI are shown in Fig. 3.

The *matK* alignment comprised 1559 characters and 381 (56%) of 682 variable sites were parsimony informative. The heuristic search of the MP analysis yielded six most parsimonious trees with a length of 1208 steps, CI = 0.732 and RI = 0.724 (cf. Table 2). The maximally supported *Anarthria* clade branches off first, followed by a split lineage containing the centrolepids and the remaining Restionaceae (1.0, 100%, 100%). One branch consists of subfamily Restionoideae (0.97, 87%, 75%). The other branch (PP 1.0, ML-BS 87%) contains subfamily Sporadanthoideae (1.0, 92%, 84%) and another very weakly supported clade (ML-BS 52%). In this clade *Gaimardia* Gaudich. of the centrolepids is sister to subfamily Leptocarpoideae (1.0, 100%, 100%).

After the exclusion of 125 characters (2192–2221, 2388–2482) the *Topo6* alignment encompassed 2452 sites. Of 522 variable characters, 263 (50%) were parsimony informative and the heuristic search provided 102 shortest trees of 911 steps long (CI = 0.772, RI = 0.703; cf. Table 2). The topology is similar to the *matK* tree, but some nodes are not or less supported. Restionoideae is monophyletic, *Gaimardia*, Sporadanthoideae and Leptocarpoideae form a polytomy. Subfamily Leptocarpoideae is not supported in this dataset.

The alignment of *PhyB* contained 1162 positions. Of 656 variable sites, 329 (50%) were parsimony informative. The heuristic search of the MP analysis yielded two most parsimonious trees with a length of 1348 steps, CI = 0.665 and RI = 0.569 (cf. Table 2). The *Anarthria* clade branches off first, followed by a clade of centrolepids and remaining Restionaceae (1.0, 85%, 100%). Within this clade *Gaimardia* is sister to all other Restionaceae (1.0, 76%, 99%). Subfamily Restionoideae is not monophyletic. Within the second lineage (0.96, 78%, 83%) *Ceratocaryum* Nees of subfamily Restionoideae branches off first. The next clade (1.0, 94%, 99%) presents the sister-group relationship of subfamilies Sporadanthoideae (0.99, 83%, 84%) and Leptocarpoideae (0.98, 71%, 79%). No cloned accessions revealed duplicate copies, suggesting that in all cases there is a single *PhyB* sequence. In *Mayaca*, where two accessions were sequenced, the results were identical.

A *Topo6* dataset of Bromeliaceae. — The *Topo6* alignment of Bromeliaceae consisted of 20 taxa (plus *Wendlandiella* Dammer of family Arecaceae chosen as the outgroup) and a total length of 1022 nucleotide positions. Of 459 variable characters, 147 (32%) were parsimony informative and 180 most parsimonious trees of 690 steps length were found (CI = 0.830, RI = 0.689; cf. Table 2). All analyses resulted in highly similar tree topologies, therefore only the 50% majority-rule consensus tree is shown (Fig. 4). *Brocchinia* Schult.f. ex Schult. & Schult.f. is sister to all remaining Bromeliaceae (MP-BS 99%), which form a polytomy. Subfamily Tillandsioideae is strongly supported (1.0, 91%, 98%), where *Glomeropitcairnia* (Mez) Mez is followed by a group (1.0, 94%, 100%) containing *Tillandsia* L. and a sister-group relationship of *Werauhia* J.R.Grant and *Vriesea* Lindl. (MP-BS 70%). *Hechtia* Klotzsch, *Puya* Molina and *Pitcairnia* L'Hér. are shown on single branches. In another lineage (0.98, 72%, 84%) *Dyckia* Schult.f. is sister to subfamily Bromelioideae (0.99, 80%, 87%). Within Bromelioideae *Fascicularia* Mez and *Bromelia* L. branch off first, followed by a polytomy (1.0, 86%, 91%), in which *Neoglaziovia* Mez, *Ursulaea* Read & Baensch and *Orthophytum* Beer appear as individual lineages. In a weakly supported group (ML-BS 58%) *Aechmea nudicaulis* Griseb. and *Billbergia* Thunb. are sisters (1.0, 82%, 79%), followed by *Neoregelia* L.B.Sm. and another very weakly supported clade (ML-BS 56%), where *Wittrockia* Lindm. is sister to a relationship of *Quesnelia* Gaudich. and *Aechmea orlandiana* L.B.Sm. (1.0, 92%, 91%).

■ DISCUSSION

Utility of *matK*, *Topo6* and *PhyB*. — The *matK* tree reported here, the plastid-based phylogeny (represented by the *rbcL*+*ndhF* tree of Bouchenak-Khelladi & al., 2014, which is based on the broadest taxonomic sampling), and the nuclear trees (McKain & al., 2016) agree broadly in resolving a graminid clade (Flagellariaceae, (Poaceae, (Ecdeiocoleaceae, Joinvilleaceae))), a restiid clade (Anarthriaceae, (Restionaceae, Centrolepidaceae)), a cyperid clade (Thurniaceae, (Juncaceae, Cyperaceae)), as well as a xyrid clade (Xyridaceae, Eriocaulaceae) that does not include Mayacaceae. *matK* and the nuclear DNA trees place

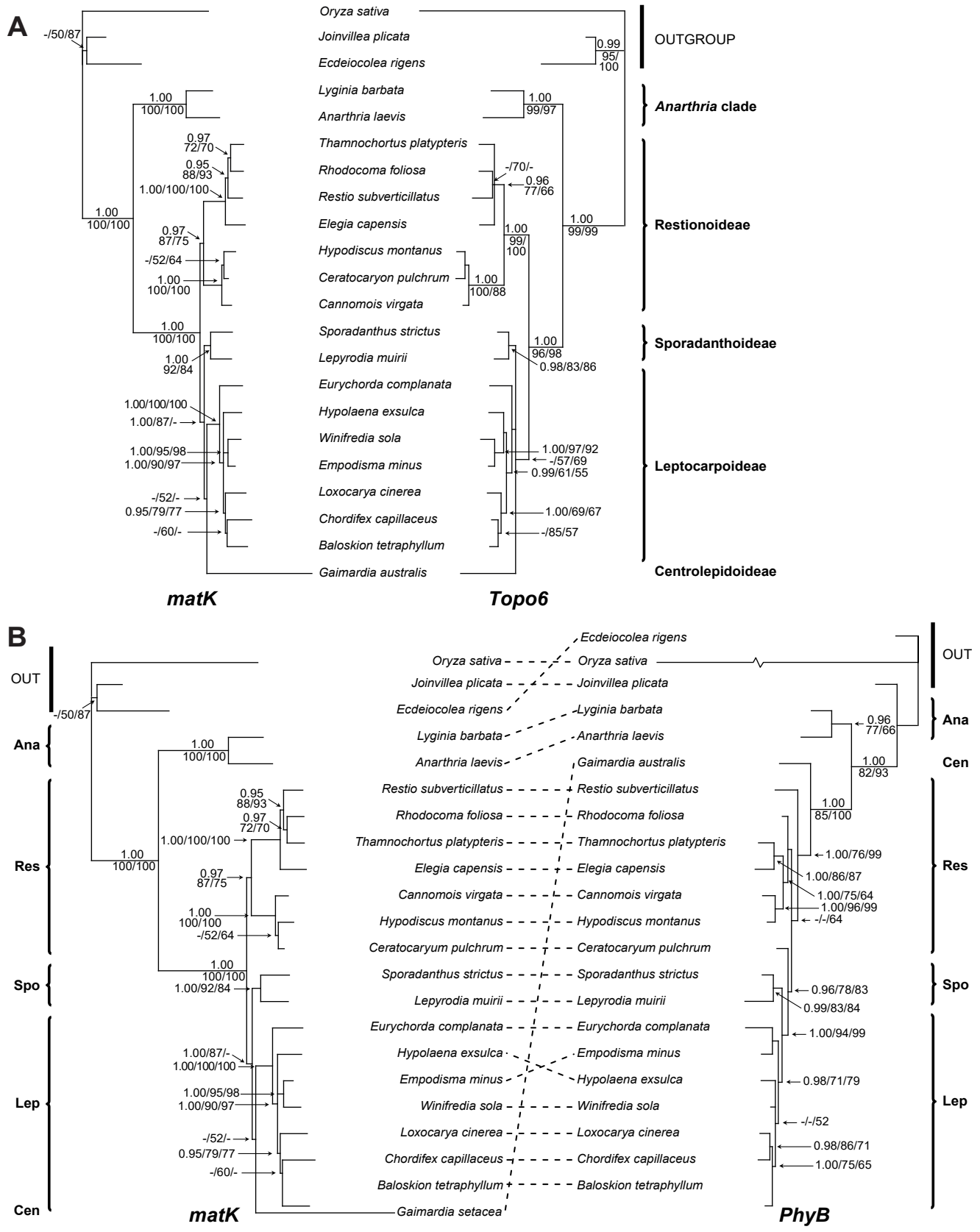


Fig. 3. A comparison of the phylogenies obtained from *matK*, *Topo6* and *PhyB* for the restiid clade of Poales. A. *matK* vs. *Topo6*. B. *matK* vs. *PhyB.* Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS).

Mayacaceae as sister to (xyrids, (restiids, graminids)), whereas *rbcL+ndhF* places the extended cyperid clade ((Mayacaceae, Rapateaceae), Thurniaceae, (Juncaceae, Cyperaceae)) in this position. Rapateaceae are either on a single branch, sister to the rest (nuclear), or in a basal polytomy (*matK*), or sister to Mayacaceae, or sister to the rest (*rbcL+ndhF*). Typhaceae is always placed basal to the Poales, but either in a clade with Bromeliaceae (*rbcL+ndhF*), or unresolved (*matK*) or as sister to Bromeliaceae and rest of Poales (McKain & al., 2016). In addition, the monophyly of all families recognized by APG IV (2016; e.g., inclusion of Anarthriaceae and Centrolepidaceae in an enlarged Restionaceae) is corroborated. Thus *matK* gives a well-resolved phylogeny of the Poales, largely consistent with the McKain & al. (2016) trees, and the plastid DNA trees of Bouchenak-Khelladi & al. (2014). Only two stem nodes are unresolved. This confirms previous work (Hilu & al., 1999; Tamura & al., 2004; Marchant & Briggs, 2007; Hardy & al., 2008; Givnish & al., 2010; Evans & al., 2015; Hertweck & al., 2015) that *matK* is a highly informative locus above the generic and at the ordinal level. Although phylogenetic accuracy benefits from additional characters or genomic regions (Knoop & Müller, 2009; Crawley & Hilu, 2012) the *matK* tree is remarkably successful in retrieving the multi-locus plastid Poales phylogeny. It also corroborates the hypothesis that the chloroplast loci result in largely congruent phylogenies. A detailed examination reveals minor discrepancies of the *matK* tree from those obtained from nuclear or multiple plastid loci. The GPWG II (2012) recognized, for example, a sister-group relationship of grass subfamilies Micrairoideae and Arundinoideae, while Fig. 1 shows Micrairoideae closely related to Danthonioideae.

The *Topo6* tree is poorly resolved with little backbone support (Fig. 2), and the only supra-family groupings supported are (Ecdeiocoleaceae, Joinvilleaceae), the cyperids (Cyperaceae, Juncaceae), and the restiids (*Anarthria* clade, Restionaceae). All families (cf. APG IV, 2016) represented by several species are retrieved as monophyletic. The most likely tree is largely congruent with the plastid and the transcriptome trees (although only 8 of 17 nodes have a posterior probability exceeding 0.95; Givnish & al., 2010; Bouchenak-Khelladi & al., 2014; Hertweck & al., 2015; Barrett & al., 2016; McKain & al., 2016), except that Xyridaceae is grouped with cyperids, rather than with Eriocaulaceae in the xyrids. The poor resolution of *Topo6* is most likely the result of the short length of the locus and consequently its relatively few informative sites.

PhyB presents a very different and unusual topology. This is not the result of the presence of paralogous copies (tested by cloning), nor from sequencing errors (tested by sequencing more accessions). There are several odd things about *PhyB*. One is that the support values often do not co-vary. This is illustrated by the Restionaceae, Cyperaceae and Juncaceae grouping, which has PP = 1.0, but no bootstrap support. This pattern is seen in several unusual groupings, such as (Mayacaceae, Typhaceae, Eriocaulaceae). An explanation could be that the posterior probability and bootstrap proportion are two fundamentally different measures of phylogenetic uncertainty (Yang & Rannala, 2005). Erixon & al. (2003), Huelsenbeck & Rannala (2004) and Yang & Rannala (2005) suggest that posterior probabilities are over-estimated when the priors are incorrect, and generally when the analysis is under-parameterized. This could suggest that *PhyB* is a complex locus, where no single model fully specifies the genetic evolution in the data. The result is that no optimal solution is found, but that this solution obtains a strong Bayesian posterior probability, whereas the bootstrap and non-parametric bootstraps indicate that these nodes are poorly supported. If we pay attention in the *PhyB* analysis only to the nodes supported by bootstrap (ML or/and MP), they are congruent with the nodes in the *Topo6* and the plastid phylogeny. This suggests that *PhyB* should be partitioned and the partitions given separate models, in order to obtain a more reliable posterior probability estimate of the phylogenetic

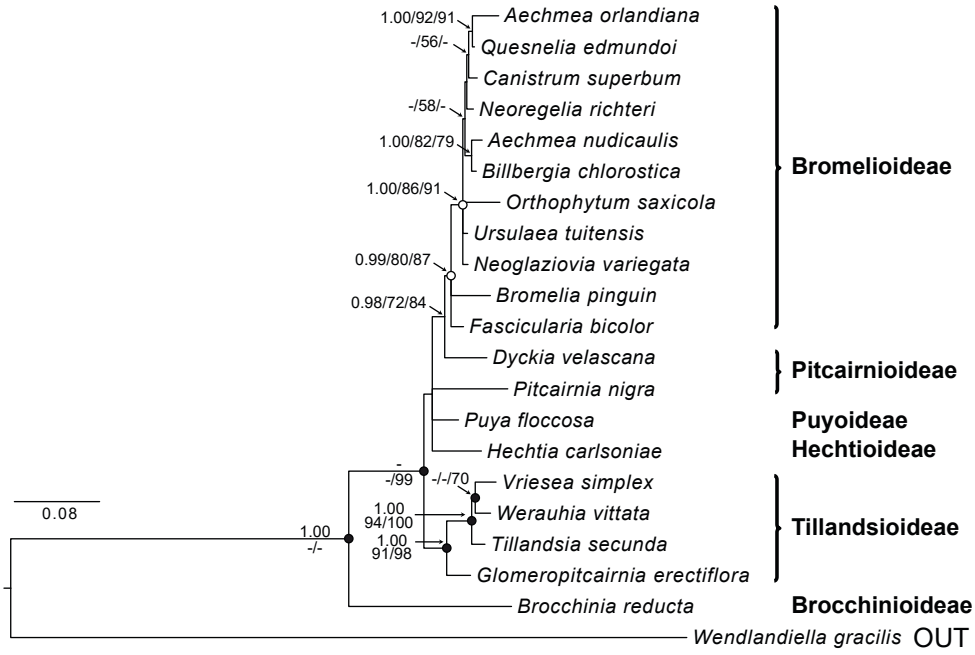


Fig. 4. Bayesian 50% majority-rule consensus tree obtained from nuclear *Topo6* (exon 8–11) DNA sequences in representatives of Bromeliaceae. Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS). The filled circles indicate the nodes also supported by the *matK* data and Givnish & al. (2014) and the empty circles indicate the nodes also supported by Givnish & al. (2014).

signal in these data, although we have not yet worked out how to accomplish this. Only three nodes receive unambiguous support from all three indices: these are (Ecdeiocoleaceae, Joinvilleaceae), (Cyperaceae, Juncaceae) and the whole of the Poales. Except for the latter, these nodes are also fully supported by *Topo6*. So, if we use all three indices, then we can correctly interpret *PhyB*. The monophyly of the families is generally supported, except for the restiids.

Poales basal group. — Our results do not contribute to solving the problem of the basal resolution of Poales, as none of the three markers had sufficient resolution. Our *matK* results (Bromeliaceae, Typhaceae, (Rapateaceae, (rest))) are congruent with ((Typhaceae, Bromeliaceae), (Rapateaceae, (rest))) reported by Hertweck & al. (2015), but not with the *rbcL* solution of (Rapateaceae, ((Typhaceae, Bromeliaceae), rest)) reported by Bremer (2002) and Davis & al. (2004), or the Bouchenak-Khelladi & al. (2014) solution of ((Typhaceae, Bromeliaceae), rest including Rapateaceae). The transcriptome analysis had the Typhaceae as first diverging, followed by Bromeliaceae (McKain & al., 2016). Some analyses also place Bromeliaceae as first diverging followed by Typhaceae (Chase & al., 2000; Soltis & al., 2000; Chase & al., 2006). The most data-rich plastome analysis (Givnish & al., 2010) also retrieved (Bromeliaceae, (Typhaceae, (rest))), and if the chloroplast genome correctly reflects the phylogeny of Poales, this is most likely the correct solution. It is evident that we do not yet have a confident resolution among these highly divergent clades. We speculate that these clades diverged within a very short time (hence the absence of a clear signal), followed by ca. 115 million years of evolution (Hertweck & al., 2015).

Bromeliaceae. — Bromeliaceae, comprising 58 genera and 3400 species (Luther, 2012), is monophyletic in all phylogenetic trees of this study (Figs 1, 2, 4). *Topo6* performance is quite variable. Several nodes are strongly supported, including the Tillandsioideae, Bromelioideae and the basal branching of *Brocchinia*. The resolution within the Tillandsioideae is also strongly supported. This corroborates the results of Givnish & al. (2011) based on plastid sequence data, and also partially the *matK* data (Fig. 1). *Topo6* does not resolve the relationships among the subfamilies. Overall, the *Topo6* marker performed very well in Bromeliaceae. The amplification and sequencing of extracted DNA from fresh leaf material was not difficult. Although with little resolution, *Topo6* could be used to test the plastid-based, as well as the nuclear sequence-based (e.g., *PHYC* or *PRK*) and combined phylogenies in Bromeliaceae (cf. Barfuss, 2012; Barfuss & al., 2016).

Mayacaceae and the xyrid clade. — Our *matK* and *Topo6* results do not resolve the position of Mayacaceae, probably due to insufficient signal. Our *PhyB* results produce the very implausible arrangement of (Poaceae, (Mayacaceae, (Typhaceae, Eriocaulaceae))). Previous studies place Mayacaceae within or closely related to the xyrid clade (Michelangeli & al., 2003; Linder & Rudall, 2005; Givnish & al., 2010), to the cyperid clade (Chase & al., 2000, 2006; Janssen & Bremer, 2004) or between these two clades (Davis & al., 2004; Hertweck & al., 2015; McKain & al., 2016). Bouchenak-Khelladi & al. (2014) group Mayacaceae with Rapateaceae as sister to the cyperids, xyrids, restiids and graminids, and McKain & al. (2016) have only Rapateaceae in this position, and place Mayacaceae as sister to xyrids, restiids and graminids. Mayacaceae is unusual in several ways. The single genus has one African species and 2–10 Neotropical species; although rooted in the ground, it can grow completely submerged. It shares a number of peculiar features with the xyrid clade (Stevenson, 1998), and thus a resolution of its phylogenetic position could be interesting.

Restiid clade. — There are two outstanding problems in the restiid clade. The first is man-made, and concerns the number of families included, and the second concerns the topological position of the Centrolepidaceae.

APG IV (2016) proposed the inclusion of Anarthriaceae and Centrolepidaceae in Restionaceae, resulting in the restiid clade consisting of only family Restionaceae. The small Western Australian *Anarthria* clade is monophyletic and sister to Restionaceae in the analyses of our three datasets (Figs. 1, 3). This is also the relationship most commonly reported (Briggs & al., 2000, 2014; Briggs & Johnson, 2000; Bremer, 2002; Michelangeli & al., 2003; Linder & Rudall, 2005; Chase & al., 2006; Bouchenak-Khelladi & al., 2014). There is consequently no phylogenetic reason to combine these two families.

The phylogenetic placement of the Australasian centrolepids (here represented by *Gaimardia*) remains difficult to determine. The clade is morphologically highly divergent, and plastid genome phylogenies have placed it on long branches, and in diverse topological positions. Our results illustrate the problem: *matK* places *Gaimardia* as sister to Restionaceae subfamily Leptocarpoideae, albeit on a long branch (Figs. 1, 3). *Topo6* places *Gaimardia* a node further down, sister to Restionaceae subfamilies Sporadanthoideae and Leptocarpoideae (Fig. 3). *PhyB* places *Gaimardia* as sister to Restionaceae s.str. (e.g., Restionoideae, Leptocarpoideae and Sporadanthoideae), consistent with the results of Michelangeli & al. (2003) and Marchant & Briggs (2007). A sister position to Sporadanthoideae has also been found, for example by Bremer (2002) and Briggs & al. (2014). Although all results place the centrolepids in or sister to Restionaceae, the precise location of the clade remains enigmatic. Briggs & al. (2014) discussed the possibility of including the centrolepids as subfamily Centrolepidoideae in an enlarged family Restionaceae, which was implemented by APG IV (2016).

Within Restionaceae three subfamilies are recognized by anatomical, morphological and molecular phylogenetic studies (Briggs & al., 2000, 2010; Linder & al., 2000, 2003; Briggs & Linder, 2009). All studies to date, including our *matK* and *Topo6*, but not *PhyB*, strongly support the monophyly of the African Restionoideae, (Figs. 1, 3) (Briggs & al., 2000, 2010, 2014; Briggs & Linder, 2009). The two tribes of this subfamily, Restioneae (inter alia *Elegia* L., *Rhodocoma* Nees, *Restio* Rottb. and *Thamnochortus* P.J.Bergius) and Willdenowieae (inter alia *Cannomois* Beauv. ex Desv., *Ceratocaryum* Nees and *Hypodiscus* Nees) (Briggs & Linder, 2009), are also strongly supported by these datasets. The *PhyB* dataset places *Ceratocaryum*, which all other datasets place in the Restionoideae, as sister to the Australian Leptocarpoideae and Sporadanthoideae; consequently, the African subfamily Restionoideae is paraphyletic.

The small subfamily Sporadanthoideae is monophyletic in all molecular phylogenetic trees (Figs. 1, 3) (Briggs & al., 2000, 2010, 2014; Briggs & Linder, 2009). The second-largest subfamily, Leptocarpoideae, is also retrieved by all current analyses, albeit without significant support in the *Topo6* tree (Figs. 1, 3). Most of our results show monotypic *Eurychorda* B.G.Briggs & L.A.S.Johnson on the basal branch of this subfamily, which supports previous investigations (Briggs & al., 2000, 2010, 2014; Briggs & Linder, 2009). The *Loxocarya* R.Br. clade is supported by the large *matK* data and *PhyB* dataset, the *Winifredia* L.A.S.Johnson & B.G.Briggs clade is confirmed by all (except *PhyB*) results (Figs. 1, 3) (Briggs & al., 2010, 2014).

■ CONCLUSIONS

The *matK* locus is very efficient at retrieving the plastid phylogeny of Poales at the family level. However, the two single-copy nuclear loci *Topo6* and *PhyB* yield highly divergent tree topologies, suggesting that combining them into a common tree would not be valid. *Topo6* shows an either perfect or near-perfect match with *matK*, suggesting that they track the same history, and implying that the plastid phylogeny could be interpreted as the taxon phylogeny. The *PhyB* data show some big mismatches with respect to both the *matK* and the *Topo6* trees. We suggest that these are due to a complex structure in *PhyB*, which might need a more complex evolutionary model.

Although more analyses did not solve the phylogenetic problems in Poales (i.e., the order of family divergence in the basal group; the placement of Mayacaceae; and the relationship between the centrolepids and Restionaceae, including the delimitation of the Restionaceae), we could establish that these are not likely due to a nuclear-plastid conflict. Even though much of the Poales phylogeny is robustly resolved and supported by both nuclear and plastid genome data, a solution to all these problems will likely require much more nuclear-encoded data.

■ AUTHOR CONSTRIBUTIONS

Study conception: AH, MR; acquisition of molecular data: AH; phylogenetic analysis: AH; drafting of manuscript and pictures: AH, HPL; critical revisions: AH, HPL, MR. All authors have read, commented and approved the final manuscript. — HPL, https://orcid.org/0000-0002-1373-2708; MR, https://orcid.org/0000-0001-5111-0945

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Appendix 1. Taxa studied for DNA sequences, provenances and GenBank accession numbers. Brief voucher information is given for sequences newly generated in this study (marked by an asterisk). Missing sequence data are indicated by dashes. GenBank accession numbers are given in the order *matK*, *Topo6* exon 8–11, *PhyB*. BG: Botanical Garden.

ARECACEAE: ***Wendlandiella gracilis*** Dammer, Peru, Ucayali, *H. Rainer*, cult. BG Halle (HAL 144533), AM114621, LT900140*, LT900113*. **BROMELIACEAE:** ***Aechmea nudicaulis*** (L.) Griseb. var. ***nudicaulis***, Dominican Republic, Paso Bajito, *A. Fläschendräger*, cult. BG Halle acc. no. 9739a (HAL 74055), –, LT900142*, –; ***A. orlandiana*** L.B.Sm., cult. BG Halle (HAL 144896), KJ580001, LT900141*, –; ***Alcantarea edmundoi*** (Leme) J.R.Grant, Brazil, Rio de Janeiro, *E. Leme*, cult. BG Halle acc. no. 9760, obtained from BG Vienna (no voucher), LT900063*, –, –; ***Billbergia chlorostica*** Saunders, Brazil, Bahia, *A. Seidel 1058*, cult. BG Halle acc. no. 6890b (HAL 144534), LT900064*, LT900143*, –; ***Brewcaria reflexa*** (L.B.Sm.) B.Holst, HQ900680, –, –; ***Brocchinia reducta*** Baker, cult. BG Halle acc. no. 9789, obtained from BG Liberec (HAL 144653), AY614018, LT900144*, LT900114*; ***Bromelia pinguin*** L., Mexico, *R. Schubert & F. Ebel XVI/43*, cult. BG Halle acc. no. 10388b (HAL 144922), JX649247, LT900145*, –; ***Canistrum superbum*** (Lindm.) Mez, Brazil, *A. Bleher 17*, cult. BG Halle acc. no. 5567 (HAL 144918), AY950025, LT900158*, –; ***Catopsis sessiliflora*** (Ruiz & Pav.) Mez, Ecuador, Mera, *A. Fläschendräger*, cult. BG Halle acc. no. 9810a (HAL 144897), LT900065*, –, –; ***Cryptanthus beuckeri*** E.Morren, AF539965, –, –; ***Deuterocohnia longipetala*** (Baker) Mez, AF162231, –, –; ***Dyckia velascana*** Mez, cult. BG Halle acc. no. 2737, obtained from G. Köhres (Germany, Erzhausen) (HAL 144920), LT905500*, LT900146*, –; ***Fascicularia bicolor*** (Ruiz & Pav.) Mez, Chile, Valdivia, *F. Kümmel*, cult. BG Halle acc. no. 9856 (HAL 144655), –, LT908911*, –; ***Fosterella micrantha*** (Lindl.) L.B.Sm., EU681860, –, –; ***Glomeropitcairnia erectiflora*** Mez, Venezuela, Isla Margarita, *A. Fläschendräger*, cult. BG Halle acc. no. 11340 (HAL 137332), AY614029, LT900147*, –; ***Greigia sphacelata*** (Ruiz & Pav.) Regel, AY950015, –, –; ***Guzmania monostachia*** (L.) Rusby ex Mez, AY949990, –, –; ***Hechtia carlsoniae*** Burt-Utley & Utley, Mexico, Iguala, cult. BG Halle acc. no. 9887, obtained from BG Utrecht (HAL 144657), AY614020, LT900148*, LT900115*; ***Hohenbergia stellata*** Schult. & Schult.f., AY950026, –, –; ***Lindmania guianensis*** (Beer) Mez, AY614019, –, –; ***Navia igneosicola*** L.B.Sm., Steyerm. & H.Rob., GU475468, –, –; ***Neoglaziovia variegata*** (Arruda) Mez, Brazil, *A. Bleher 14*, cult. BG Halle acc. no. 4418 (HAL 144921), AY950051, LT900149*, –; ***Neoregelia richteri*** W.Weber, Brazil, *A. & M. Bleher*, cult. BG Halle acc. no. 9931 (HAL 103699), LT900066*, LT900150*, –; ***Nidularium procerum*** Lindm., AY950013, –, –; ***Orthophytum saxicola*** (Ule) L.B.Sm., Brazil, *A. Bleher 74*, cult. BG Halle acc. no. 9955a (HAL 144526), JX649269, LT900151*, –; ***Pitcairnia nigra*** (Carrière) André var. ***nigra***, Ecuador, Mindo, *A. Fläschendräger*, cult. BG Halle acc. no. 11102 (HAL 138769), LT900067*, LT900152*, –; ***Puya floccosa*** (Linden) E.Morren ex Mez, Venezuela, Gran Sabana, *A. Fläschendräger*, cult. BG Halle acc. no. 10884 (HAL 144654), LT900068*, LT900153*, LT900116*; ***Quesnelia edmundoi*** L.B.Sm. var. ***edmundoi***, Brazil, Rio de Janeiro, cult. BG Halle acc. no. 9983, obtained from BG Heidelberg acc. no. 103155 (HAL 137503), AY950046, LT900154*, –; ***Racinaea schumanniana*** (Wittm.) J.R.Grant, Ecuador, Mera, *A. Fläschendräger*, cult. BG Halle acc. no. 9994 (HAL 144895), LT900069*, –, –; ***Tillandsia secunda*** Kunth, Ecuador, Ibarra, *A. Fläschendräger*, cult. BG Halle acc. no. 10240 (HAL 144919), LT900070*, LT900155*, –; ***Ursulaea tuitensis*** (Magaña & E.J.Lott) Read & Baensch, Mexico, Jalisco, *Böhme*, cult. BG Halle acc. no. 12388, obtained from BG Bonn acc. no. 95 1072/1 - ZSS 070105 (HAL 144656), –, LT908912*, –; ***Vriesea simplex*** (Vell.) Beer, Dominican Republic, Salto de Jimenoa, *A. Fläschendräger*, cult. BG Halle acc. no. 10371a (HAL 144522), LT900071*, LT900156*, –; ***Werauhia vittata*** (Mez & Wercklé) J.R.Grant, Costa Rica, vulcano Poas, *K. Horn*, cult. BG Halle acc. no. 8206 (HAL 14454), LT900072*, LT900157*, LT900117*. **CYPERACEAE:** ***Blysmus compressus*** (L.) Panz. ex Link, KJ513577, –, –; ***Bolboschoenus maritimus*** (L.) Palla, cult. BG Halle (HAL 144528), LT900073*, LT900162*, –; ***Carex conferta*** Hochst. ex A.Rich., KP083048, –, –; ***C. medwedewii*** Leskov, Georgia, Mtskheta-Mtianeti, Stepantsminda, *S. Gebauer 1307/34* (HAL), –, LT900160*, LT900118*; ***Cladium mariscus*** (L.) Pohl, Switzerland, Wallis, *M. Röser 1463* (HAL), LT900074*, –, –; ***Cymophyllus fraserianus*** (Ker Gawl.) Kartesz & Gandhi, KP273711, –, –; ***Cyperus diffusus*** Vahl, cult. BG Halle (HAL 144531), LT900075*, –, LT905501*; ***C. macrocarpus*** (Kunth) Boeckeler, cult. BG Halle (HAL 144937), LT900077*, LT900161*, –; ***Cypringlea analecta*** (Beetle) M.T.Strong, KJ513594, –, –; ***Eleocharis palustris*** (L.) Roem. & Schult., cult. BG Halle (HAL 144527), LT900076*, –, –; ***Eriophorum vaginatum*** L., KJ513615, –, –; ***Isolepis aucklandica*** Hook.f., KJ513621, –, –; ***Kobresia esenbeckii*** (Kunth) Noltie, KP273712, –, –; ***Lepidosperma laterale*** R.Br., Australia, New South Wales, *M. Röser 10878 & al.* (HAL), LT900078*, –, –; ***Machaerina rubiginosa*** (Spreng.) T.Koyama, Australia, New South Wales, *M. Röser 10882 & al.* (HAL), LT900079*, –, –; ***Mapania palustris*** (Hassk. ex Steud.) Fern.-Vill., KP083067, –, –; ***Oreobolopsis tepalifera*** T.Koyama & Guagl., KJ513623, –, –; ***Phylloscirpus deserticola*** (Phil.) Dhooge & Goetgh., KJ513624, –, –; ***Schoenoplectus tabernaemontani*** (C.C.Gmel.) Palla, cult. BG Halle (HAL 144530), LT900080*, –, LT900119*; ***Schoenoxiphium lehmannii*** (Nees) Kunth ex Steud., KP273715, –, –; ***Scirpodendron ghaeri*** (Gaertn.) Merr., AB088804, –, –; ***Scirpus sylvaticus*** L., KJ513654, –, –; ***Trichophorum alpinum*** (L.) Pers., KJ513656, –, –; ***Uncinia banksii*** Boott, KJ513666, –, –; ***Zameioscirpus muticus*** Dhooge & Goetgh., KJ513668, –, –. **ECDEIOCOLEACEAE:** ***Ecdeiocolea monostachya*** F.Muell., Australia, Western Australia, *B.G. Briggs 9522* (NSW 613172), DQ257528, LT900163*, LT900120*; ***E. rigens*** B.G.Briggs, Australia, Western Australia, *B.G. Briggs 9707* (NSW 745909), GQ409048, LT900164*, LT900110*; ***Georgeantha hexandra*** B.G.Briggs & L.A.S.Johnson, DQ257531, –, –. **ERIOCAULACEAE:** ***Eriocaulon decangulare*** L., KJ772761, –, –; ***E. scariosum*** Sm., Australia, New South Wales, *M. Röser 10923 & al.* (M. Röser private herb.), –, LT900165*, LT900122*; ***E. septangulare*** With., AY952430, –, –; ***Leiothrix flavescens*** (Bong.) Ruhland, Brazil, Bahia, *E.B. Souza 1005* (NY 01112551), LT900081*, LT900166*, LT900121*; ***Syngonanthus flavidulus*** (Michx.) Ruhland, KJ773197, –, –. **FLAGELLARIACEAE:** ***Flagellaria indica*** L., cult. BG Halle (HAL 8151), AB040214, LT900167*, LT900123*. **JOINVILLEACEAE:** ***Joinvillea plicata*** (Hook.f.) Newell & B.C.Stone, cult. BG Halle (HAL 144938), DQ257535, HG793958, LT900111*. **JUNCACEAE:** ***Juncus articulatus*** L., cult. BG Halle (HAL 144525, HAL 144535), KP083050, –, LT900124*; ***Luzula campestris*** (L.) DC., lawns in BG Halle (no voucher), LT900083*, LT900168*, –; ***L. sylvatica*** (Huds.) Gaudin, cult. BG Halle (HAL 144532), LT900082*, –, –; ***Marsippospermum grandiflorum*** (L.f.) Hook., Chile, Antartica Chilena Province, Isla Gordon, *J.M. Bonifacino 4178* (NY 1680445), LT900084*, –, –; ***Rostkovia magellanica*** (Lam.) Hook.f., Chile, Antartica Chilena Province, Isla Gordon, *J.M. Bonifacino 4190* (NY 1680457), LT900085*, –, –. **MAYACACEAE:** ***Mayaca fluviatilis*** Aubl., Venezuela, Roraima, *G.J. Hoogenstrijd*, cult. BG Halle acc. no. 8572 (HAL 144529), KP083052, LT900169*, LT900125*. **MUSACEAE:** ***Musa velutina*** H.Wendl. & Drude, cult. BG Halle acc. no. 8628a, obtained from BG Bogor (HAL 144658), GQ374868, LT900170*, LT900126*. **POACEAE:** ***Anomochloa marantoidea*** Brongn., AF164381, HG793948, AF137291; ***Aristida purpurea*** var. ***wrightii*** (Nash) Allred, U.S.A., Texas, *D.S. Seigel & J.E. Ebinger 15241* (NY 1632760), HG794004, HG793994, LT900127*; ***Arundo donax*** L., cult. BG Halle (HAL 144850), HG793998, HG793940, LT900128*; ***Brachypodium distachyon*** (L.) P.Beauv., AM234568, NC_0161 34.1, LN554539; ***Chasmanthium latifolium*** (Michx.) H.O.Yates, cult. BG Halle (HAL 82959), HG794003, HG793941, LT900129*; ***Danthonia decumbens*** (L.) DC., Germany, Saxony, *M. Röser 10659* (M. Röser private herb.), –, LT908913*, –; ***D. spicata*** (L.) Roem. & Schult., AF164409, –, AF137299; ***Fargesia nitida*** (Mitford) Keng f. ex T.P.Yi, cult. BG Halle (HAL 105157), HG794002, HG793989, LT900130*; ***Hordeum marinum*** subsp. ***gussoneanum*** (Parl.) Thell., AB078108, –, –; ***Isachne albens*** Trin., China, Guizhou, *B. Bartholomew & al.* (NY), HG794006, HG793996, LT900131*; ***Leersia oryzoides*** (L.) Sw., Germany, Saxony, *H. Boyle & al.* (HAL 144935), CBM42989, HG793959–HG793965, LT900132*; ***Miscanthus sinensis*** Andersson, cult. BG Halle (HAL 89739), HG793999, HG793942, LT900133*; ***Oryza sativa*** L., cult. BG Halle (no voucher), HG794000, HG793980, LT900094*; ***Pharus lappulaceus*** Aubl., HG794007, HG793992, AF137321; ***Pleioblastus fortunei*** (Van Houtte) Nakai, cult. BG Halle (HAL 144862), HG793997, HG793949, LT900134*; ***Poa lepidula*** (Nees & Meyen) Soreng & L.J.Gillespie, FR694884, HG793982, LN554568; ***Puelia olyriformis*** (Franch.) Clayton, HQ604000, –, –; ***Sorghum bicolor*** (L.) Moench, AF164418, XM002459 909, AF182394; ***Streptochaeta angustifolia*** Soderstr., AF164382, HG793990, AF137328. **RAPATEACEAE:** ***Rapatea* sp.**, AF539958, –, –; ***Spathanthus unilateralis*** (Rudge) Desv., French Guiana, *S.A. Mori 25867* (NY 866067),

Appendix 1. Continued.

LT900086*, LT900171*, LT900135*; ***Stegolepis* sp.**, KP083054, –, –. **RESTIONACEAE:** ***Alexgeorgea ganopoda*** L.A.S.Johnson & B.G.Briggs, KF181911, –, –; ***Anarthria gracilis*** R.Br., KF206011, –, –; ***A. laevis*** R.Br., Australia, Western Australia, *B.G. Briggs 9843* (NSW 757762), KF206013, LT900138*, LT900092*; ***Anthochortus graminifolius*** (Kunth) H.P.Linder, KF452375, –, –; ***Aphelia brizula*** F.Muell., DQ257500, –, –; ***Apodasmia brownii*** (Hook.f.) B.G.Briggs & L.A.S.Johnson, KF181913, –, –; ***Askidiosperma rugosum*** E.Esterhuysen, AY881484, –, –; ***Baloskion tetraphyllum*** (Labill.) B.G.Briggs & L.A.S.Johnson, cult. BG Halle (HAL 144514), AF164379, LT900172*, LT900095*; ***Calorophus elongatus*** Labill., DQ257502, –, –; ***Cannomois virgata*** (Rottb.) Steud., cult. BG Halle (HAL 144863), LT900087*, LT900173*, LT900096*; ***Catacolea enodis*** B.G.Briggs & L.A.S.Johnson, GQ409049, –, –; ***Centrolepis monogyna*** (Hook.f.) Benth., DQ257505, –, –; ***C. strigosa*** (R.Br.) Roem. & Schult., DQ257507, –, –; ***Chaetanthus leptocarpoides*** R.Br., KF181915, –, –; ***Chordifex capillaceus*** B.G.Briggs & L.A.S.Johnson, Australia, Western Australia, *B.G. Briggs 9454* (NSW 461256), GQ409030, HG793954, LT900097*; ***Ceratocaryum pulchrum*** H.P.Linder, South Africa, Western Cape Province, *H.P. Linder 7389* (Z), KF452499, LT900174*, LT900098*; ***Cytogonidium leptocarpoides*** (Benth.) B.G.Briggs & L.A.S.Johnson, KF181916, –, –; ***Desmocladus confertospicatus*** (Steud.) B.G.Briggs, KF181887, –, –; ***D. elongatus*** B.G.Briggs & L.A.S.Johnson, GQ409044, –, –; ***Elegia capensis*** (Burm.f.) Schelpe, cult. BG Halle (HAL 144516), LT900088*, LT900175*, LT900099*; ***Empodisma minus*** (Hook.f.) L.A.S.Johnson & D.F.Cutler, Australia, New South Wales, *M. Röser 10881 & al.* (HAL), DQ257513, LT900177*, LT900100*; ***Eurychorda complanata*** (R.Br.) B.G.Briggs & L.A.S.Johnson, Australia, Tasmania, *B.G. Briggs 9136* (NSW 264949), DQ257514, HG793956, LT900101*; ***Gaimardia australis*** Gaudich., Chile, Antartica Chilena Province, Isla Hoste, *J.M. Bonifacino 4270* (NY 1686586), –, LT900159*, LT900109*; ***G. fitzgeraldii*** F.Muell. & Rodw., DQ257516, –, –; ***G. setacea*** Hook.f., DQ257517, –, –; ***Hopkinsia anoectocolea*** (F.Muell.) D.F.Cutler, DQ257518, –, –; ***Hypodiscus montanus*** Esterh., South Africa, Western Cape Province, *H.P. Linder 7298* (Z), KF452469, LT900176*, LT900102*; ***Hypolaena exsulca*** R.Br., Australia, Western Australia, *B.G. Briggs 9959* (NSW 869862), KF206006, LT900178*, LT900103*; ***Leptocarpus canus*** Nees, GQ409045, –, –; ***Lepyrodia muirii*** F.Muell., Australia, Western Australia, *B.G. Briggs 9443* (NSW 422602), KF206007, LT900179*, LT900104*; ***Loxocarya cinerea*** R.Br., Australia, Western Australia, *B.G. Briggs 9574* (NSW 714459), GQ409047, LT900180*, LT900105*; ***Lyginia barbata*** R.Br., Australia, Western Australia, *B.G. Briggs 9872* (NSW 783042), DQ257523, LT900139*, LT900093*; ***Mastersiella spathulata*** (Pillans) H.P.Linder, KF452505, –, –; ***Platycaulos major*** (Mast.) H.P.Linder, AY881538, –, –; ***Platychorda rivalis*** B.G.Briggs & L.A.S.Johnson, Australia, Western Australia, *B.G. Briggs 9438* (NSW 422597), KF206014, LT908914*, –; ***Restio subverticillatus*** (Steud.) Mast., cult. BG Halle (HAL 144934), KF452391, LT900181*, LT900106*; ***Rhodocoma foliosa*** (N.E.Br.) H.P.Linder & C.R.Hardy, cult. BG Halle (HAL 144898), AY640392, LT900182*, LT900107*; ***Soroveta ambigua*** (Mast.) H.P.Linder & C.R.Hardy, KF452500, –, –; ***Sporadanthus strictus*** (R.Br.) B.G.Briggs & L.A.S.Johnson, Australia, Western Australia, *B.G. Briggs 9817* (NSW 756781), KF181923, LT900183*, LT900112*; ***Staberoha distachyos*** (Rottb.) Kunth, AY881544, –, –; ***Thamnochortus platypteris*** Kunth, South Africa, Western Cape Province, *H.P. Linder 7480* (Z), AY690738, LT900184*, KC514020; ***Tremulina tremula*** (R.Br.) B.G.Briggs & L.A.S.Johnson, DQ257527, –, –; ***Willdenowia arescens*** Kunth, KF452376, –, –; ***Winifredia sola*** L.A.S.Johnson & B.G.Briggs, Australia, Tasmania, *T. Entwisle 3332* (NSW 713239), GQ409021, LT900185*, LT900108*. **THURNIACEAE:** ***Prionium* sp.**, KP083053, –, –; ***Thurnia* sp.**, KP083068, –, –. **TYPHACEAE:** ***Typha* × *glauca*** Godr., U.S.A., Wisconsin, *M. Nee 59372* (NY), LT900089*, LT900186*, LT900136*; ***T. latifolia*** L., AB088801, –, –; ***T. laxmannii*** Lepech., cult. BG Halle (HAL 144936), LT900090*, –, –; ***Sparganium erectum*** L., JQ435570, –, –. **XYRIDACEAE:** ***Abolboda* sp.**, KP083058, –, –; ***Xyris difformis*** Chapm., cult. BG Halle acc. no. 8845, obtained from BG Dresden (HAL 144521, HAL 144659), LT900091*, LT900187*, LT900137*.

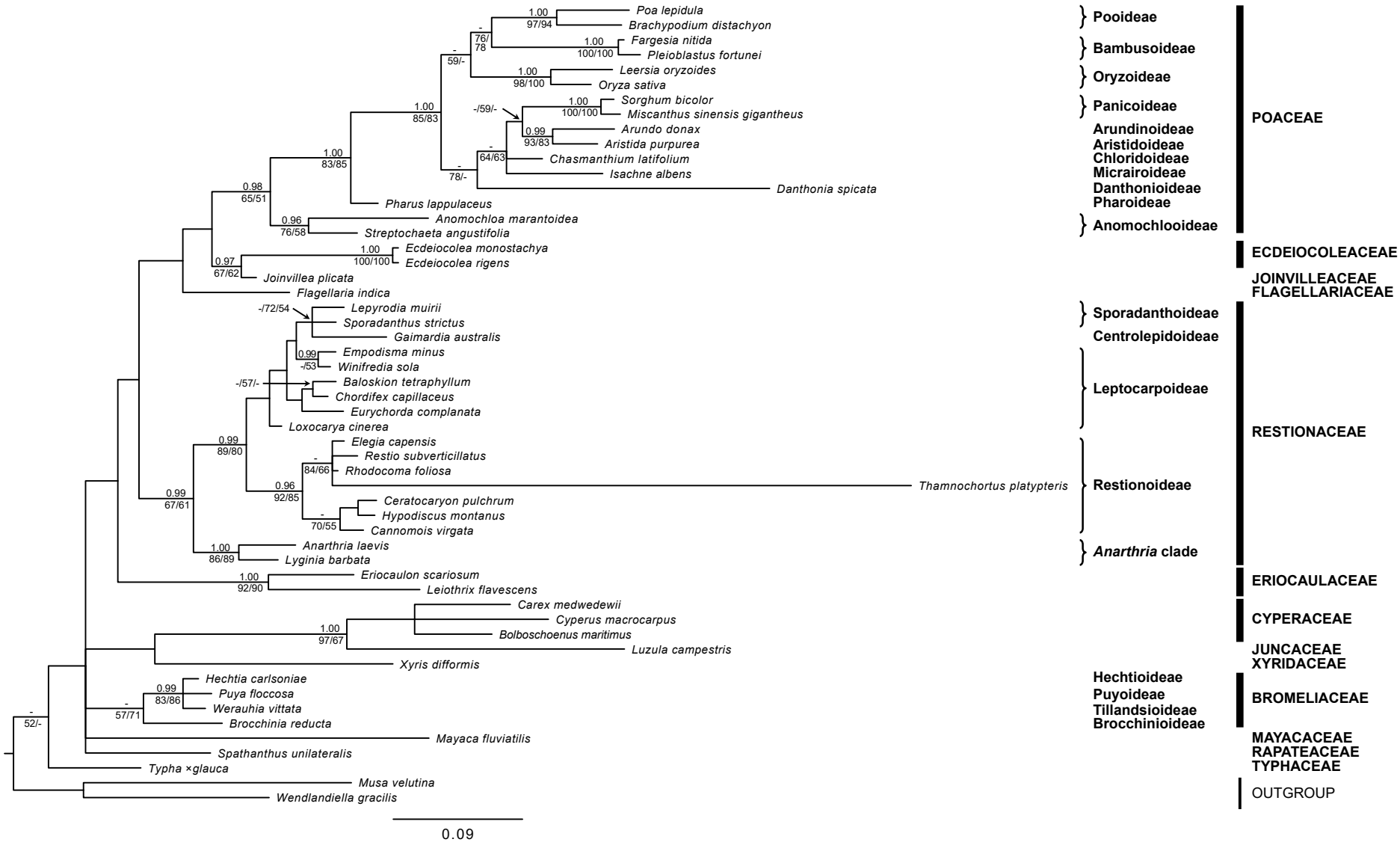

Fig. S1. Bayesian 50% majority-rule consensus tree obtained from nuclear *Topo6* (exon 8–11) DNA sequences in representatives of Poales. Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS).

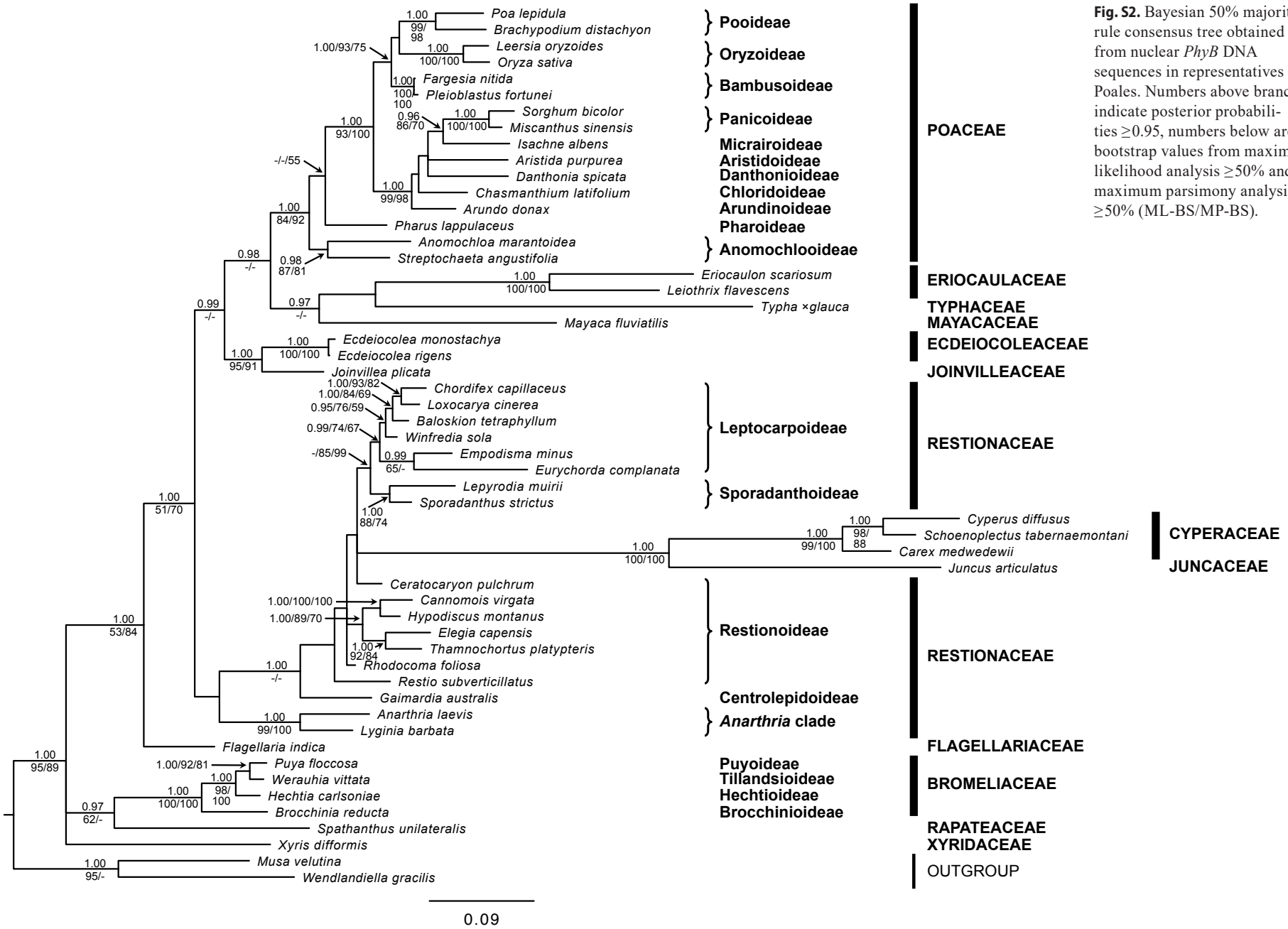

Fig. S2. Bayesian 50% majority-rule consensus tree obtained from nuclear *PhyB* DNA sequences in representatives of Poales. Numbers above branches indicate posterior probabilities ≥0.95, numbers below are bootstrap values from maximum likelihood analysis ≥50% and maximum parsimony analysis ≥50% (ML-BS/MP-BS).